REVIEW ARTICLE

Oxygen deprivation stress in a changing environment

R.M.M. Crawford[1,3] and R. Braendle[2]

[1] Plant Science Laboratory, Sir Harold Mitchell Building, The University, St Andrews, Fife KY16 9AL, UK

[2] Pflanzenphysiologisches Institut der Universität Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

Abstract

Past research into flooding tolerance and oxygen shortages in plants has been motivated largely by cultivation problems of arable crops. Unfortunately, such species are unsuitable for investigating the physiological and biochemical basis of anoxia-tolerance as selection has reduced any tolerance of anaerobiosis and anaerobic soil conditions that their wild ancestors might have possessed. Restoration of anoxia-tolerance to species that have lost this property is served better by physiological and molecular studies of the mechanisms that are employed in wild species that still possess long-term anoxia-tolerance. Case studies developing these arguments are presented in relation to a selection of crop and wild species. The flooding sensitivity and metabolism of maize is compared in relation to rice in its capacity for anaerobic germination. The sensitivity of potato to flooding is related to its disturbed energy metabolism and inability to maintain functioning membranes under anoxia and post-inoxia. By contrast, long-term anoxia-tolerance in the American cranberry (Vaccinium macrocarpon) and the arctic grass species Deschampsia beringensis can be related to the provision and utilization of carbohydrate reserves. Among temperate species, the sweet flag (Acorus calamus) shows a remarkable tolerance of anoxia in both shoots and roots and is also able to mobilize carbohydrate and maintain ATP levels during anoxia as well as preserving membrane lipids against anoxic and post-anoxic injury. Phragmites australis and Spartina alterniflora, although anoxia-tolerant, are both sulphide-sensitive species which can pre-dispose them to the phenomenon of die-back in stagnant, nutrient-rich water. Glyceria maxima adapts to flooding through phenological adaptations with a seasonal metabolic tolerance of anoxia confined to winter and spring which, combined with a facility for root aeration and early spring growth, allows rapid colonization of sites with only shallow flooding. The diversity of responses to flooding in wild plants suggests that, depending on the life strategy and habitat of the species, many different mechanisms may be involved in adapting plants to survive periods of inundation and no one mechanism on its own is adequate for ensuring survival.

Key words: Anoxia, hypoxia, flooding, Zea mays, Solanum tuberosum, Oryza sativa, Acorus calamus, Phragmites australis, Glyceria maxima, cranberry.

Introduction

World-wide increases in flooding, milder, wetter winters and mounting levels of pollution together with eutrophication of flood waters, highlight the need for reducing the long-term damage caused by soil inundation both to cultivated crops and natural vegetation. The increased canalization of major rivers such as the Rhine and the resultant flooding of the bordering lowlands has at last forced public attention on the necessity of using natural flood-tolerant plant communities to provide a buffer zone to reduce flooding intensity, even at the risk of some low level of flooding of agricultural land. The universal observation that oxygen deprivation, either completely (anoxia) or partially (hypoxia), is detrimental to most species of higher plants inevitably raises the question of whether there are any fundamental physiological differences between plants in their cellular responses to imposed anaerobiosis. It is often assumed, given the generality that most cultivated plants avoid, rather than

---

[3] To whom correspondence should be addressed. Fax: +44 1334 463366.

tolerate oxygen shortages, that facilitation of oxygen supply in adverse conditions is a more profitable area for scientific enquiry (Armstrong *et al.*, 1994). Research into flooding tolerance (one of the most frequent, but not the only cause of oxygen shortage) has been motivated largely by cultivation problems of arable crops. Such investigations have been dominated by the desire to elucidate the primary causes of injury in sensitive agricultural species (Jackson *et al.*, 1990; Kozlowski, 1984). This emphasis is to be expected, given that arable farming is, in general, incompatible with prolonged flooding. However, this dependence on drainage for plant productivity may be of historical origin due to the Neolithic origins of agriculture in the drought-prone lands of the near and middle east. The high productivity of many fresh and even salt-water marshes suggests that more attention should be given to understanding the adaptations of wild plants to the consequences of flooding.

The research that has been carried out on flooding injury (largely with crop species) has paid particular attention to investigating the physiological and morphological reactions of roots to flooding (Koncalova, 1990; Laan *et al.*, 1991; Saglio *et al.*, 1988). It might be thought logical to assume that active growing roots are the relevant organs for the study of flooding injury as they have to endure the risk of oxygen deprivation as a result of inundation. However, when the relative anoxia-tolerance of roots of different species is compared it is generally found that oxygen deprivation is fatal to all roots, even in species with anoxia-tolerant perennating organs such as rhizomes or tubers. In wetland species, there are no known cases of any prolonged survival over weeks or months of absorptive roots entirely deprived of oxygen (Crawford, 1992). However, just because the absorbing roots have died does not necessarily imply that all parts of the plant in question are equally sensitive to oxygen deprivation. Not all the organs of any plant have to survive oxygen deprivation for the plant to be considered anoxia tolerant. It is sufficient to achieve anoxia-tolerance if those parts that serve regeneration of the whole plant survive prolonged oxygen depletion.

The basic philosophy of agriculture historically, has been to adapt conditions to suit plants. Therefore, avoidance of flooding by adequate drainage has been the most effective method of reducing flood-injury. In relation to the adaptations of the species under cultivation, avoidance mechanisms have also claimed most attention in research into flooding survival. Thus, the development of aerenchyma and hormonal adjustments to plant growth that are initiated by high water tables have been extensively investigated (Armstrong *et al.*, 1994). Avoidance of flooding stress is also aided through the life-strategies of most agricultural species. The majority of arable crops are annual plants which are naturally programmed to die after flowering. Consequently, crops that have been

selected for agriculture are usually derived from ancestors that would not have been subjected to regular flooding. The plants most at risk are the winter cereals and pulses (peas and beans). Once established, temporary flooding is usually not a problem for small seedlings due to the proximity of the root system to the base of the leaves and, therefore, minimal distances for the diffusion of oxygen from shoot to root. However, during germination the initial stages can be severely damaged by soaking injury in maize and many pulse species (Powell and Matthews, 1978; Pretorius and Small, 1991). As a result of successful breeding programmes, maize cultivars have been selected that adapt well to the growing season of many central and even northern European countries provided that no flooding occurs in spring. However, the germination sensitivity of even modern maize varieties to flooding, as compared with the greater tolerance of barley and winter wheat, still remains (Plate 1).

The problem of poor field-emergence in some pulse and cereal crops as a result of soaking injury after sowing may be increasing in many parts of the world due to climatic change resulting in a greater frequency of heavy periods of rainfall particularly in winter and spring. All models of global climatic change predict increased winter soil moisture in northern regions, partly due to increased precipitation and also through more frequent snow-melt throughout the winter and spring seasons (Houghton *et al.*, 1990). Paradoxically, climatic warming may also be a disadvantage to crops grown in sub-arctic regions as mild weather can replace accustomed snowfall with rain falling on frozen ground which results in prolonged periods of ice-encasement. When grasslands and winter crops are encased in ice the danger of anoxia intolerance becomes severe (Gudleifsson, 1994; Gudleifsson and Larsen, 1993).

Changed climatic conditions and modern technology expose what might be expected as flood-resistant species to dangers of oxygen deprivation. The use of heavy machinery for the cutting of reeds in winter is leading, in many cases, to a decline in reed growth due a combination of soil compaction together with shoot proliferation from frequent cutting reducing overwintering rhizome reserves (Ostendorp, 1987*a*). Changes in flooding frequency, possibly as a result of climatic change, can also reduce growth in certain circumstances even of such a flood-tolerant species as *Phragmites australis* (Ostendorp, 1987*b*).

Current developments in plant molecular genetics and biotechnology provide an opportunity for expanding the traditional approach to researching anoxia-tolerance and its relevance to overcoming problems of flooding injury. Given that the genetic basis of whatever anoxia-tolerance the ancestors of our present crop species may have possessed was probably lost in the early stages of Neolithic crop selection, there remains now the alternatives of either engaging in the difficult and time-consuming search for

Plate 1. Flooding damage in a maize field: maize seedlings in the depression were killed after heavy rainfall just after sowing. Barley (left) and wheat (right) were not affected. (Photo R. Braendle.)

more tolerant ancestors of our crop species (if they can still be found) or else exploiting the techniques of molecular biology to create new genetically manipulated species with increased anoxia-tolerance.

Irrespective of whether or not genetic manipulation is being employed in increasing anoxia-tolerance, it is necessary to focus attention on those parts of the life cycle that expose plants to tissue damage should the oxygen supply fail. In annual crops this is the seed. In perennial species, the greater size, density, complexity, and distance of tissues from a source of oxygen creates a hindrance in its supply to overwintering organs. In perennial plants the consequences of oxygen shortages have, therefore, to be considered in relation to the successful survival from one season to the next and how buried rhizomes and tubers or tap and sinker roots respond to periods of prolonged oxygen deprivation.

The above differences in survival strategy result in different perceptions of the hazards of flooding as seen by agriculturists, working with a genetic stock selected for maximum yield in a stress-protected environment, as compared with ecologists, interested in maintaining the biodiversity of natural populations able to withstand periodic stresses and climatic oscillations. In agricultural research, flooding problems are usually investigated in relation to their impact on the current growing season. Some agricultural crops are also at risk outside their main

growing season as in the winter cereals and oilseed rape. However, with natural vegetation and with perennial crops there is a greater cumulative risk from winter and spring flooding and, therefore, a need to understand the varying ability of plants to withstand flooding outside the growing season (Crawford, 1992).

The aim of this review is to highlight the variety of responses that can be found in wild plants in relation to flooding. Despite decades of such research into flooding injury in crop plants, there is still no evidence of any common lethal target in flood-sensitive species. By turning attention to flood-tolerant wild species this review attempts to show the diversity of responses to flooding that are to be found in wetland vegetation. Depending on the life strategy and habitat of the species, many different mechanisms can be involved with no one mechanism on its own being adequate to ensure survival.

Assessing anoxia and hypoxia tolerance

The inherent variability of all natural systems is also met in relation to levels of anoxia-tolerance in wetland plant communities. Depending on the time of year and the condition of the perennating organ, anoxia-tolerance can vary from a few days to several months (Table 1). In bog and marsh plants tolerance of oxygen-deprivation is usually greater in winter and early spring and decreases to a

Table 1. *Maximun anoxia-tolerance of underground stems and rhizomes as observed in an anaerobic incubator at 20–25°C*

Survival was measured in having an ability to grow new shoots after the period of anaerobic incubation. Data adapted in part from Braendle and Crawford (1987) and Crawford *et al.* (1994a). Lower field soil temperatures of 5–10°C would extend the length od anoxia-tolerance 2–3 times.

Group 1. Underground stems with minimal tolerance of anoxia, surviving only 1–4 d	
Solanum tuberosum	1–2 d
Eriophorum vaginatum	2–4 d
Saxifraga hieracifolia	2–4 d
Saxifraga cernua	2–4 d
Oxyria digyna	2–4 d

Group 2. Underground stems with increased tolerance of anoxia, surviving 4–21 d	
Carex rostrata	4 d
Mentha aquatica	4 d
Juncus effusus	4–7 d
Juncus conglomeratus	4–7 d
Eriophorum angustifolium	5–7 d
Saxifraga oppositifolia	7–10 d
Saxifraga caespitosa	7–14 d
Ranunculus repens	7–9 d
Iris germanica	7–10 d
Eleocharis palustris	7–12 d
Carex papyrus	7–14 d
Filipendula ulmaria	7–14 d
Tussilago farfara	7–14 d
Phalaris arundinacea	9–15 d
Glyceria maxima	7–21 d
Festuca vivipara (Iceland)	18–25 d

Group 3. Underground stems with a high tolerance of anoxia, surviving 1–3 months	
Iris pseudacorus	>28 d
Spartina anglica	>28 d
Phragmites australis	>28 d
Typha latifolia	>28 d
Schoenoplectus lacustris	>35 d
Deschampsia beringensis (N. Alaska)	>80 d
Scirpus maritimus	>90 d
Acorus calamus	>90 d

minimum after shoot expansion and carbohydrate depletion at the beginning of the growing season (Crawford, 1992; Haldemann and Braendle, 1986). With roots there is also great variability, but on a much shorter time scale from a few hours to days. In some crop species, for example, cotton and soybean, root death from anoxia can take place in as little as 3 h and 5 h, respectively, with a proportion of the roots being killed within 30 min (Huck, 1970). Consequently, when comparisons are being made on the relative anoxia-tolerance of different species or cultivars it is possible to have considerable confusion as to what is meant by anoxia-tolerance. In experiments to ascertain the role of alcohol dehydrogenase activity (ADH) on anoxia-tolerance in maize roots (Roberts *et al.*, 1984), plants which were deficient in ADH activity were compared with normal plants and found to die

within 12 h, whereas normal plants lasted 24 h. Similar genetic attempts to determine which Adh genes are necessary for anoxic survival in maize (Lemke-Keyes and Sachs, 1989) were also based on plants that were all dead (tolerant and intolerant) after 24 h of anoxia. Although possibly useful in studying enzyme systems, such short-term differentiation of anoxia-tolerance is hardly sufficient to label one variety ecologically anoxia-tolerant and the other intolerant. On a slightly longer time scale, all these strains could be more realistically classified as intolerant. Any suggestions as to how anoxia-tolerance is achieved from this type of observation can not be automatically extended to cover organs such as *Iris* and *Acorus* rhizomes, where anoxia-tolerance is measured in months. A similar confusion exists when rice is used as a yardstick to measure tolerance of oxygen shortage. Rice seeds can germinate without oxygen, but further development of root and shoot depends on access to oxygen (Perata and Alpi, 1993). The tolerance of the rice seedlings to total oxygen deprivation varies greatly with the cultivar and environmental conditions (Perata *et al.*, 1992), but the tolerance of anoxia, even in the most flood-resistant varieties of rice, is much less than that of shoots developing from rhizomes of anoxia-tolerant marsh species such as *Iris pseudacorus*, *Typha latifolia* and *Schoenoplectus lacustris* (Barclay and Crawford, 1982; Braendle, 1990; Braendle and Crawford, 1987).

Anoxia-tolerance is not a constant property which varies only with the nature of the species. Many situations have been recorded where the ability of plants to withstand an oxygen shortage varies with the time of year and the state of the carbohydrate reserves in the perennating organs (see the case-study of *Glyceria maxima*). Geographical origin can also play a role, as with populations of *Saxifraga oppositifolia* from high arctic sites in Spitsbergen which are able to endure prolonged periods of ice-encasement in the field. In the laboratory these high Arctic populations also show greater tolerance of anoxia than more southerly populations of the same species from Scotland (Crawford *et al.*, 1994a). Other factors such as pollution, and harvesting of shoots (as in reed cutting) can also have a modifying effect on the ability of wetland plants to overwinter in oxygen-deficient habitats. A detailed examination of some of these examples is given below in the section on biodiversity in relation to oxygen deprivation.

## Contributing factors to damage from oxygen-deprivation

Causes of anoxic and post-anoxic injury

Some confusion in studies on plant behaviour under anaerobic stress arises from the fact that there are many ways of dying as a result of oxygen deprivation. So far

no single, universal cause of anoxic death has been found for plant tissues. In some cases, as in maize seedlings, irreversible injury to cells takes place rapidly, in a matter of hours, apparently due to metabolic dysfunction causing an impairment of membrane function, leading to an immediate onset of cytoplasmic acidosis (Roberts *et al.*, 1985*a*, *b*). The role of cytoplasmic pH in triggering a rapid switch to ethanol production within an hour of the onset of anoxia (Fig. 1) has been convincingly demonstrated in maize by using ^{31}P nuclear magnetic resonance (Fox *et al.*, 1995). In other cases irreversible damage takes place somewhat later (1–2 weeks) and is accelerated when the tissues are returned to air by the onset of post-anoxic injury when rapid peroxidative damage is brought about by active radical generation and toxic oxidative products such as acetaldehyde (Albrecht and Wiedenroth, 1994; Crawford, 1992; Pfister-Sieber and Braendle, 1994; Studer and Braendle, 1988; Van Toai and Bolles, 1991).

Earlier controversies as to whether or not ethanol is toxic to oxygen-deprived plant tissues (Crawford, 1967;

Jackson *et al.*, 1982) can now be resolved by following the sequence of events that takes place when tissues that have been incubated anaerobically are restored to air. Ethanol, when produced in an anaerobic tissue, will take part in the sequence of reactions that follows when the post-anoxic oxidation of reduced iron triggers off the generation of active oxygen species (superoxide etc.), followed by hydrogen peroxide through the action of superoxide dismutase (SOD) (Elstner and Osswald, 1994).

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^-$$

$$O_2^- + O_2 \text{—SOD} \rightarrow H_2O_2.$$
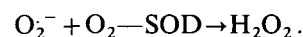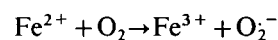
Ethanol that has accumulated under anoxia can then react with hydrogen peroxide and provide a rapid generation of acetaldehyde

$$C_2H_5OH + H_2O_2 \text{—Catalase} \rightarrow CH_3CHO + 2H_2O.$$

Hydrogen peroxide generated in the initial stages of post-anoxia allows catalase to carry out a rapid oxidation of ethanol to produce a surge of acetaldehyde. This sequence of events causes ethanol which has been accumulated during a period of anaerobiosis to be potentially damaging to cell membranes due to its rapid oxidation to acetaldehyde on re-exposure to air. Merely exposing aerobic roots to external ethanol solutions, therefore, does not mimic the real situation and is not a relevant measure of ethanol toxicity as a result of post-flooding injury. Species with long-term tolerance of anoxia with an ethanolic fermentation (see *Acorus calamus* below) avoid an excessive accumulation of ethanol by diffusive loss of ethanol from the rhizome into the surrounding medium (Studer and Braendle, 1987).

Many rhizomatous species from wetland habitats are naturally well-defended against post-anoxic injury by a variety of defence mechanisms which can be grouped under two headings, (1) enzymatic and (2) anti-oxidant. Enzymatic protection against post-anoxic injury has been studied in the overwintering tubers of the anoxia-tolerant species *Iris pseudacorus* and compared with the intolerant *I. germanica*. The tolerant species differs from the intolerant in being able to increase its superoxide dismutase (SOD) activity during a month-long period of anaerobic incubation and thus enters the post-anoxic phase well-equipped to counteract the potential hazards of superoxide generation (Monk *et al.*, 1987). There appears to be much variation in wetland species as to which type of anti-oxidant activity is present in their perennating organs (Wollenweber-Ratzer and Crawford, 1994). Many species appear to rely on anti-oxidants such as ascorbic acid, α-tocopherol and glutathione. These defences are also dependent on enzymatic activity as ascorbic acid and glutathione are only active as anti-oxidants in the reduced
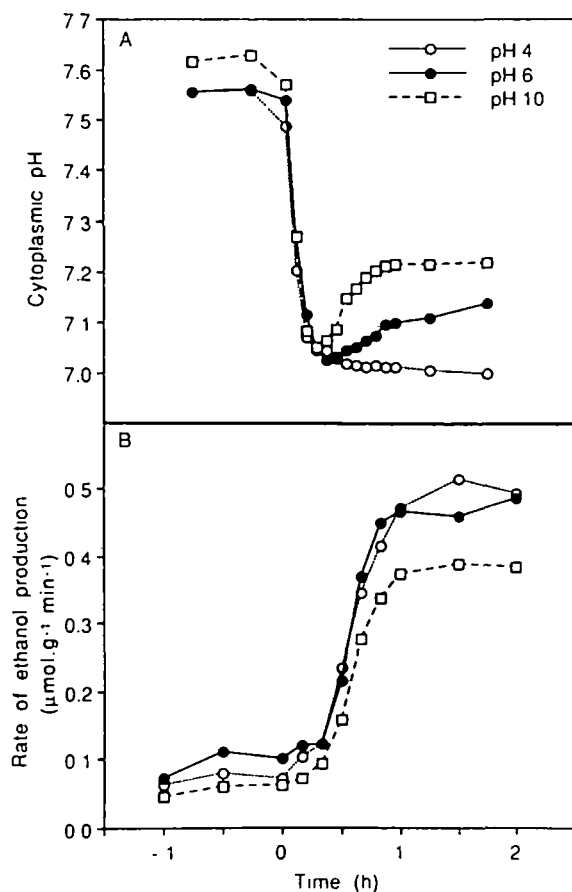

Fig. 1. Graphs showing the effect of a switch from aerobic to anaerobic conditions on the cytoplasmic pH (A) and the rate of ethanol production (B) for anoxia-intolerant maize root tips bathed in a medium either at pH 4 (O), pH 6 (●) or pH 10 (□). The switch from oxygen to nitrogen occurred at time zero, and each curve is the mean of three separate experiments. (Reproduced with permission from Fox *et al.*, 1995.)

state. Ascorbic acid is a common biological antioxidant and its role in counteracting post-anoxic injury in plants has been demonstrated for *Cicer arietinum* L. (Crawford and Wollenweber-Ratzer, 1992). Glutathione and ascorbate occur in millimolar concentrations in the plant cell, where in the reduced form they are active as antioxidants. Perfusion of anoxic *Iris germanica* rhizomes with 5 mM ascorbic acid just before re-exposure to air (Fig. 2) can reduce the amplitude of the electro-paramagnetic signal generated by active radical production (Crawford *et al.*, 1994*b*). There is strong evidence that the cellular glutathione and ascorbate levels increase as a response to phytotoxic oxidative processes and that high levels of

antioxidants confer resistance to oxidative cell damage (Alscher, 1989). The enzymes dehydroascorbate reductase (DHAR; EC 1.8.5.1) and monodehydroascorbate reductase (MR; EC 1.6.5.4) are involved in the cellular defence against phytotoxic oxidation by maintenance of the reduced active forms of glutathione and ascorbate, respectively: MR catalyses the reduction of monodehydroascorbate (MDA) to ascorbate and DHAR plays an important role in the H_2O_2 detoxification system (Foyer and Halliwell, 1976). In soybean (*Glycine max*) short-term anoxia results in the production of superoxide radicals, while longer treatments induce high levels of superoxide dismutase activity and thus provide a greater protection when the plants return to air (Van Toai and Bolles, 1991). By contrast, the defence system against post-anoxic injury in rice appears weaker as it is activated only when the plants return to air (Ushimaru *et al.*, 1992). However, this may be related to the ever-present danger of excessive uptake of iron by flooded plants (see below) leading to increased exposure to active radical generation (Hendry and Brocklebank, 1985).

Direct and indirect causes of death from oxygen shortage

The distinction between anoxic and post-anoxic injury gives a metabolic basis for explaining in part the differences found in many plant tissues in their loss of viability when deprived of oxygen. In roots of highly intolerant species such as soya bean and tomato, death takes place in hours and probably reflects the collapse of membrane stability with fall in ATP and energy charge levels. In other species the length of time spent under anoxia will affect the outcome of their re-exposure to air. Prolonged anoxia at high temperatures depletes carbohydrate reserves and allows the accumulation of metabolites that will cause injury when oxidized on return to air. To the oxidation of ethanol discussed above can be added the surge in ethylene production produced as a result of the oxidation of ACC. Although ethylene is not toxic to plants it can induce potentially maladaptive changes in plant form. Prolonged exposure to an oxygen-deficient soil will expose roots to potentially toxic concentrations of reduced ions such as Mn^{2+} and Fe^{2+}. Normally, plants in wetland habitats will be defended against the uptake of these ions by oxidizing them at the root surface from surplus supplies of oxygen diffusing downwards from the shoot, or in the case of some rhizomatous species transported actively by humidity-induced pressure flow (Armstrong *et al.*, 1992; Grosse *et al.*, 1991). When the supply of oxygen is interrupted, then these defence mechanisms fail and poisoning from toxic metal ions takes place. Excessive absorption of iron under flooded conditions can subsequently lead to tissue damage through enhanced active radical production (Hendry and Brocklebank, 1985).
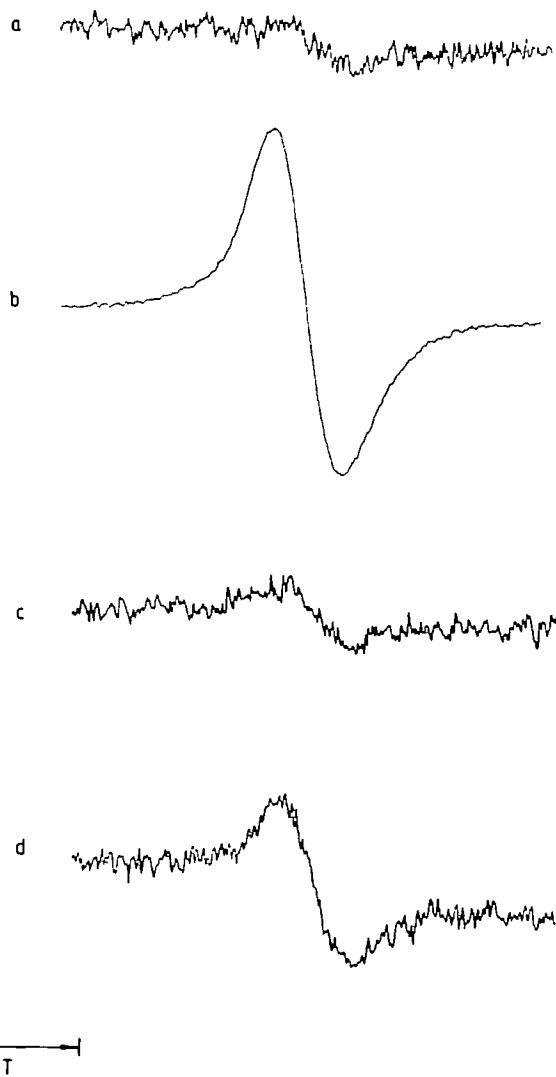

Fig. 2. Electron spin resonance signals showing the post-anoxic induction of active radicals in rhizomes of *Iris germanica* The graphs shows first derivative 9.3 GHz spectra from solid samples (*c*. 0.02 g) from aerobic (a) and post-anoxic (b) rhizomes of *Iris germanica*. A reduction in amplitude of the EPR-signal with 5 mM ascorbic acid and 5 mM glutathione is represented in lines (c) and (d). (mT = milliTesla). (Reproduced with permission from Crawford *et al.*, 1994*b*.)

Oxygen deprivation in crop plants

Rice and maize

Comparisons of anoxia-tolerance in cereals show very considerable differences at the time of germination. Most cereals fail to germinate when deprived of oxygen, but rice is the exception. The ability of rice to germinate anaerobically comes from a capacity to extend the coleoptile and once this reaches a source of oxygen, root growth and leaf expansion begin. It is therefore in the initiation of seedling development that rice differs from other cereals. Carbohydrate availability is needed to sustain active fermentation and comparison of rice with wheat, barley, oats, and rye shows that only rice is able to synthesize a-amylase under anoxia. The inhibitory effect of anoxia on a-amylase induction appears to be due to the inability of cereal seeds (with the exception of rice) to respond to gibberellic acid under conditions of oxygen deprivation (Perata *et al.*, 1993). Once germination has taken place, the ability of rice to survive in flooded soils comes from its capacity to avoid oxygen deficits. The capacity for aeration of the root system through aerenchyma development is ten times greater in rice than barley and four times that found in maize (Perata and Alpi, 1993).

Potato

Potato cultivars are universally sensitive to oxygen shortage and to reduce the risk of flooding are normally cultivated in ridges. A common practice in the eighteenth and nineteenth centuries in the hyperoceanic countries of Ireland and western Scotland was the 'lazy bed' tradition of cultivation in which extra wide furrows were constructed to extend the growing season which is severely limited by winter soil saturation. In potato, it has been shown that energy metabolism and related processes are highly sensitive to oxygen depletion and that under anoxia ATP production at 0.18 μmol ATP g^{-1} fr. wt. h^{-1} is not sufficient to maintain membrane function on a long-term basis, although the tubers are supplied with ample starch reserves. Energy charge values decrease continuously with increasing periods of oxygen deficiency and adenylate pools are simultaneously exhausted. Furthermore, the production of considerable amounts of lactic acid lowers cytoplasmic pH (Sieber and Braendle, 1991). Cytoplasmic acidosis caused by the inhibition of ATPases in the tonoplast and accumulation of lactic acid could be responsible for the early cell death that is found under anoxia in potato tubers. Additionally, the lack of ATP induces membrane damage (Pfister-Sieber and Braendle, 1994). Free fatty acids increase in anoxic tissues, membranes become leaky and the cells lose their electrolytes. There is apparently a narrow threshold zone between normal membrane function and membrane disintegration

indicated by a small increase in free fatty acid concentration that is indicative of whether or not the membranes are in a leaky or non-leaky condition (Fig. 3). This situation is aggravated on re-aeration as lipid peroxidiation is evident from the appearance of malonedialdehyde and ethane (Pfister-Sieber and Braendle, 1994). Thus, potato tubers not only suffer severe metabolic dysfunction under anoxia, but are highly susceptible to the additional stress of post-anoxic injury on return to air.

The American cranberry

The American cranberry (*Vaccinium macrocarpon* Ait.) is one of very few crops to be cultivated commercially in bogs. Although noted from an early date by American settlers as a fruit worth collecting, it was not until the early decades of the nineteenth century that the first attempts were made in New England to cultivate this species in specially managed bogs (Eck, 1990). Cranberry vines are susceptible to winter frosts and it was found that flooding the bogs could protect the plants from the dangers of frost desiccation. However, when the bogs are iced over, and then subsequently covered in snow, the shading of the evergreen cranberry vines was observed to cause the death of stems and terminal buds. These symptoms were found to be a diagnostic feature of an oxygen-deficiency syndrome which was subsequently shown to be directly related to the carbohydrate status of the overwintering vines (Bergman, 1953). Any factors which deplete the overwinter reserves of carbohydrate, such as poor summers or heavy cropping can make the vines susceptible to oxygen shortages when flooded and can
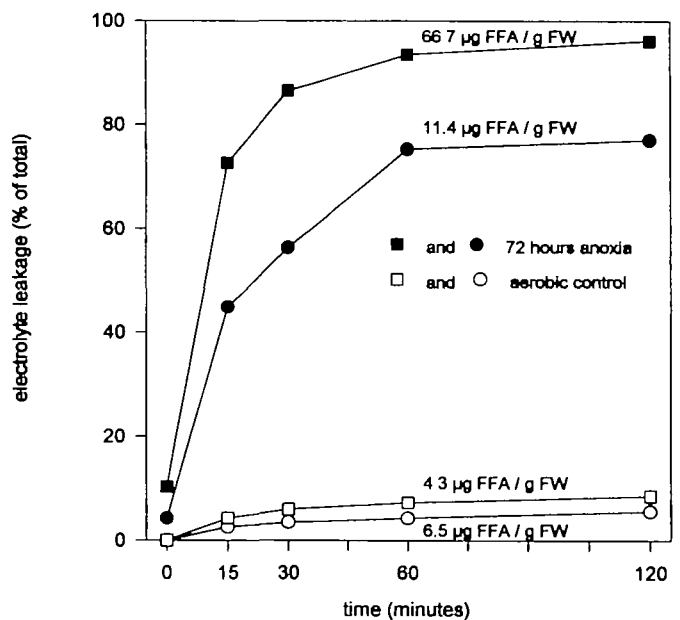

Fig. 3. Solute leakage and free fatty acid concentration of anoxic potato tissues (2 replicates O, □). Note the minimal difference between the occurrence of free fatty acids between leaky and non-leaky tissues.

severely limit the use of inundation as a method for reducing winter frost-desiccation injury. Any means which can be used either to produce higher carbohydrate reserves or to increase the oxygen content of the flood-water can reduce the onset of the oxygen-deficiency symptoms (for review see Eck, 1990).

Biodiversity in relation to oxygen deprivation

For too long, short-term economic reasons have made crop plants the central object of study in flooding tolerance research. With current agricultural over-production more land is being set aside for purposes other than the production of annual arable crops. Increased use is likely to be made in the future of permanent and perennial plant communities for an ever wider range of purposes including wood production, sewage purification, eutrophication control, and flood-management. Such ecologically-oriented uses of wetland communities are to be welcomed if they are managed in such as way as to preserve the diversity and homeostatic properties of the plant and animal communities. Fluctuating water tables, which hitherto have not been tolerated due to the practical needs of arable farming, are less hazardous to managed wetlands and more consideration can be given to the use flood-tolerant communities where overwintering oxygen shortages are a high probability.

Proper management of wetland areas needs to pay detailed attention to the diversity of tolerance mechanisms that exist in plants of wetland habitats if biodiversity is to be preserved. In natural habitats where water tables

are allowed to fluctuate, regeneration of a wide range of flood-tolerant species is facilitated (Crawford, 1992) and leads to the development of diverse communities within the marsh or bog. Examination of their distribution relative to each other shows, in many cases, marked developmental sequences which have often been discussed in relation to plant succession. However, behind this ecological succession there lies a wealth of variation in relation to the various physical, phenological and metabolic adaptations that plants use to overcome oxygen shortages. When such fluctuations do not take place there is a tendency for only a limited range of species to survive due to reduced habitat variation. Constant flooding also limits reproduction to those species that reproduce vegetatively (Crawford, 1992). Consequently, in marshes with a steady regime of shallow flooding, with nutrient rich water, the vegetation can become dominated by nutrient-demanding competitive species such as *Glyceria maxima* to the detriment of other species (Studer-Ehrensberger *et al.*, 1993). Ecological management of such areas needs to be aware of the diversity of mechanisms that are involved in tolerance of anoxia and other wetland stresses (Fig. 4). Within any one wetland area there will be a variety of mechanisms which individually adapt the species to flooding tolerance and collectively promote the biodiversity of the wetland habitat.

Wetland species with divergent responses to oxygen depletion

When a selection of species living in aquatic and marsh habitats is tested for anoxia-tolerance, the capacity of the
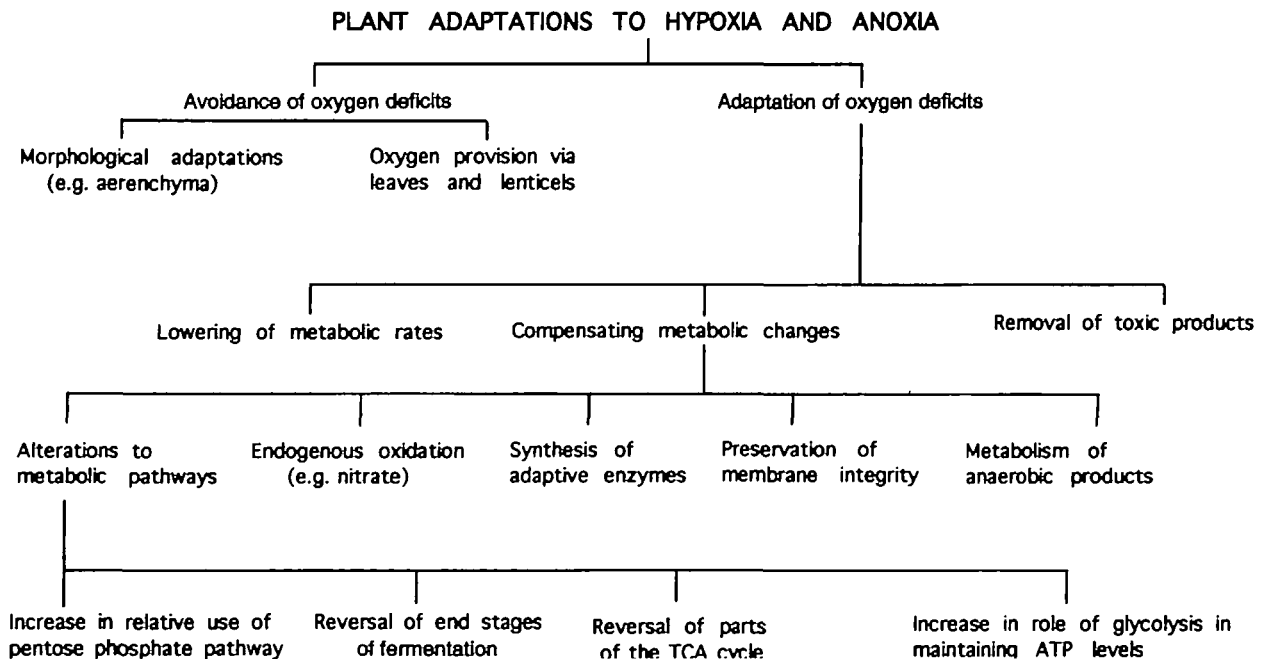

PLANT ADAPTATIONS TO HYPOXIA AND ANOXIA

Fig. 4. Diagrammatic representation of the diversity of adaptations that can be observed in higher plants which can contribute to their tolerance of flooding.

overwintering (perennating) organs to survive is found to be vary variable. In some species, such as bulrush (*Schoenoplectus lacustris*) and cat's-tail (*Typha latifolia*) tolerance of anoxia is extremely well developed. Not only can the rhizomes survive several weeks without any oxygen, they can also extend new shoots (Braendle and Crawford, 1987; Crawford, 1989). As a result of prolonged tolerance of anoxia, submerged rhizomes that have lost last season's dead stalk, which acts as a snorkel connecting them with a supply of air (Brix, 1989), can still emerge in spring from an entirely oxygen-deficient habitat. Other species, such as the common reed (*Phragmites australis*) and meadow sweet (*Filipendula ulmaria*) can survive long periods without any oxygen, but only begin shoot extension once the oxygen supply is restored (Barclay and Crawford, 1982).

Table 2. *Polar lipid and free fatty acid content* (n = 5) *under air and under 70 d anoxia in rhizomes of* Acorus calamus *and 14 d anoxia in* Iris germanica

Data from Henzi and Brändle (1993).

	Lipids (μg g^{-1} fr. wt.)		Fatty acids (μg g^{-1} fr. wt.)	
	Control	Anoxia	Control	Anoxia
Acorus calamus	695 ± 44	651 ± 38	< 10	< 20
Iris germanica	891 ± 37	425 ± 29	< 10	138 ± 17

The sweet flag (Acorus calamus)

Acorus calamus is a non-fertile (n = 36, triploid) neophyte, probably introduced from one plant (Schröter, 1908) that belonged in 1574 in Vienna to the famous herbalist Charles de L'Ecluse (latinized Clusius 1525–1609). Nevertheless, despite this apparent lack of population heterozygosity this species is a competitive and aggressive invader of heterotrophic European lake edges. This invasive success appears to stem in part from the extreme tolerance of anoxia found in the rhizomes of this species. The rhizomes have high carbohydrate reserves throughout the year (Haldemann and Braendle 1986) which are more than sufficient to sustain ethanolic fermentation for several months. Furthermore, ATP production is considerably greater than in potato (1.55–2.33 μmol ATP g^{-1} fr.wt. h^{-1}) and adenylate pools and energy charge levels remain stable and high throughout prolonged periods of anoxia (Sieber and Braendle, 1991). These figures indicate a state of equilibrium between ATP production and consumption coupled with metabolic rates that are high enough to allow an extended viability for tissues under anoxia. In addition, the porous nature of the rhizomes and their position on the surface of lake muds allows excess ethanol to diffuse out into the lake water and avoids the dangers of post-anoxic conversion to acetaldehyde discussed above (Studer and Braendle, 1987). Messenger RNAs for glycolytic and glycolysis related
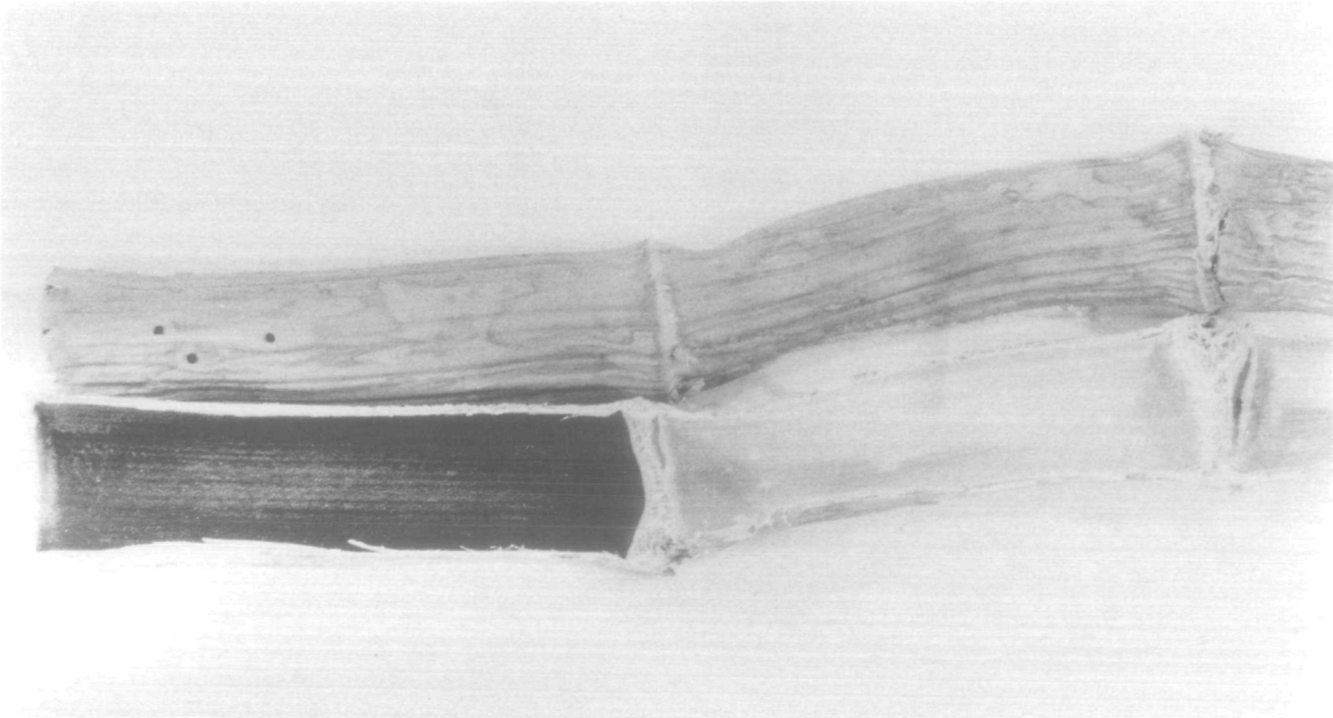

Plate 2. Effect of grazing by the larvae of the reed beetle *Donacia claviceps* on rhizomes of *Phragmites australis* in the presence of sulphide. Above; exterior of rhizome showing left an internode region with holes caused by larval grazing and below; interior of the same rhizome showing damage resulting from access of sulphide due to larval grazing. (Photo. R. Braendle.)

enzymes are induced anaerobically under artificial anoxia as well as in the natural habitat during winter (Bucher *et al.*, 1995; Bucher and Kuhlemeier, 1993) The proteins formed under anoxia in the laboratory and the field have been shown to be highly active. Moreover, in contrast to non-tolerant species, many additional proteins other than those involved directly in anaerobic metabolism are also synthesized under field and laboratory anoxia and clearly indicate that oxygen depletion is less of a metabolic perturbation and less likely to lead to cellular dysfunction in this species than in potato (Armstrong *et al.*, 1994).

In addition, rhizomes of *A. calamus* are able to store and detoxify nitrogen that has been taken up as ammonium by the roots and transferred into alanine. The main storage compound in winter rhizomes is the nitrogen-rich amino acid arginine (Haldemann and Braendle, 1986, 1988). Arginine is readily converted into transport amino acids in spring when growth starts (Weber and Braendle, 1994). Nitrogen recycling and continuous uptake favours growth and development and protein synthesis in this
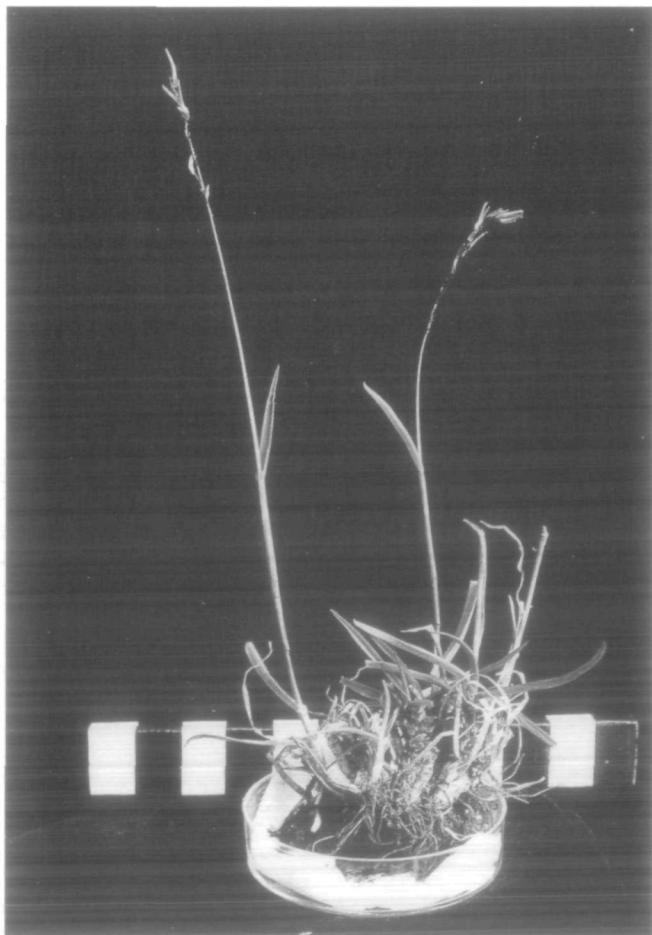

Plate 3. Intact plant of *Poa arctica* R.Br. in Spitsbergen which has been kept under total anoxia for 7 d at ambient temperature (4–7 °C) in total darkness and re-exposed to air for 12 h. Note the lack of any visible symptoms of injury and the complete turgidity of the leaves and flowering stalk. (Photo R. Crawford.)

species in comparison to other marsh plants. A similar strategy is used for the detoxification and utilization of sulphide formed in anaerobic soils. It is stored in the rhizomes as glutathione (Weber and Braendle, 1996). Glutathione is used as a sulphur source, but can also serve in the anti-oxidative defence mechanisms, in addition to α-tocopherol and phenolics (Larson, 1988).

The most outstanding strategy with regard to anoxia-tolerance in this species is probably the stability of membrane lipids under anoxia and their protection against peroxidation damage when the tissues are re-aerated (Henzi and Brändle, 1993). After 70 d of anoxia, lipids show only minimal alterations to the saturation level with the principal change being a shift in fatty acid saturation from 18:3 linolenic acid to 18:2 linoleic acid. Furthermore, free fatty acids in the tissues are minimal and there is little evidence of membrane breakdown (Table 2). By comparison, *Schoenoplectus lacustris* rhizomes show injury after 35 d of anoxia while lipids of *Iris germanica* are fully saturated after 14 d if not already in a state of disintegration. Moreover, in the rhizomes there is only a minor production of the peroxidation products malonedialdehyde and ethane. The membranes of this species are clearly well-adapted to withstand prolonged periods of oxygen deprivation. Lipid metabolism under anoxia differs from that of proteins in that lipids are preserved while proteins can be synthesized *de novo*. This distinction is not unexpected given that lipids will require desaturases and molecular oxygen for their synthesis. *Acorus calamus* is, therefore, particularly well-defended against the dangers of anoxia, both in terms of internal resistance to anoxia and resistance to externally generated anaerobic products in the soil solution. In this way *A. calamus* is better adapted than even such anoxia-tolerant species as *Phragmites australis* and *Spartina alterniflora* where high sulphide concentration can be damaging (see below).

Glyceria maxima

Among amphibious plant species, *Glyceria maxima* represents an important example of an ecological strategy for wetland survival, namely seasonal tolerance of anoxia in early spring. Despite this seasonal variation in anoxia-tolerance, *G. maxima* is able to out-compete more anoxia-tolerant species such as *Filipendula ulmaria* (Studer-Ehrensberger *et al.*, 1993) due to its capacity for early spring growth enabling it to pre-empt the occupation of sites by other later developing species. In summer *G. maxima* rhizomes are not as tolerant of anoxia as some other wetland species and can not survive prolonged deep flooding. During the aestival period, the tolerance of detached rhizomes to total anoxia is less than one week. When deprived of oxygen at high summer temperatures (22 °C) the rhizomes can lose 50 per cent of their

Plate 4. Intact plant of *Saxifraga caespitosa* L. of in Spitsbergen which has been kept under total anoxia for 7 d at ambient temperature (4–7 °C) in total darkness and re-exposed to air for 12 h. Note the lack of any visible symptoms of injury, the turgidity of the leaves and the retention of the flower. (Photo R. Crawford.)

total non-structural carbohydrate reserves in 4 d (Barclay and Crawford, 1983). In this case, the underlying component of anoxia-intolerance is energy starvation. By contrast, overwintering rhizomes survive up to 3 weeks at 22 °C in the laboratory and doubtless even longer under field conditions. The physiological basis for this seasonal dependence of anoxia-tolerance is not fully understood, but may be due in part to high carbohydrate reserves in winter coupled with a less active metabolism of the overwintering organs.

In sites where reed die-back is associated with abnormally high mineral nutrient concentrations *Glyceria maxima* can be used as a successful replacement species. Planted into such seemingly phytotoxic sites, as seen from the decline of *Phragmites australis*, cuttings of *Glyceria maxima* are able to produce new roots within a few days.

The survival strategy of *Glyceria maxima* in wetland sites is that of a stress-avoider rather than a stress-tolerator. The species has a powerful capacity for oxygen transfer from shoots to roots and the well-developed aerenchyma is protected against accidental flooding by the presence of subdivisions of longitudinally arranged cells. This arrangement prevents inundation of the whole gas lacunae in event of any accidental physical damage and, therefore, reduces the risk of a sudden anoxic stress developing in the underground organs (Armstrong *et al.*, 1994). The capacity for flooding tolerance in this species, however, is strictly limited and the species is not a suitable replacement for *Phragmites* in areas with large fluctuations in the levels of the water table. The short and soft leaves of *Glyceria maxima* decay readily and thus fail to provide a snorkel for prolonged periods of submergence.

Phragmites australis

The common reed *Phragmites australis*, in common with *Acorus calamus*, and *Spartina alterniflora* (also wetland geophytes) combines all year round anoxia-tolerance with a high capacity for oxygen transport from shoots to roots during the growing season and these species can be considered as both avoiders and toleraters of the anoxic stress condition of wetland sites. Nevertheless, despite this two-pronged tolerance of inundation stress both *Phragmites australis* and *Spartina alterniflora* can suffer from elevated levels of sulphide (Bradley and Morris, 1990; Fürtig *et al.*, 1996; Koch *et al.*, 1990; Koch and Mendelssohn, 1989; Pearson and Havill, 1988; Pezeshki *et al.*, 1988). High sulphide concentrations, above about 1 mM, occur frequently in reduced sediments of eutrophic lakes, polluted area and in estuarine muds. An extensive study of sulphide tolerance in *Phragmites australis* showed that sulphide applied under hypoxic conditions (<0.6 ppm oxygen) severely affects root energy metabolism of reed plants with weakly developed shoots mainly by inactivation of metallo-enzymes. Normally sulphide has no direct access to rhizome tissues because of the thickened rhizome surface. However, grazing by the larvae of the reed beetle (*Donacia claviceps*) can create lesions (Plate 2) which cause flooding of the rhizome air-spaces with sulphide rich water (Ostendorp, 1993). Sulphide can be translocated into the rhizome where it is partially detoxified by the formation of glutathione. The intermediate compounds are cysteine and glutamylcysteine with *o*-acetlyserine acting as the sulphide acceptor. Rhizomes are less sensitive to sulphide poisoning than roots, but the detoxification capacity is limited and sulphide accumulates. The following phenomena have been observed in roots, (1) a decrease in adenylate energy charge and total adenylates, (2) a decrease of alcohol dehydrogenase activity, and (3) a decrease in post-hypoxic respiratory capacity (Fürtig *et al.*, 1996).

Anoxia-tolerance in high arctic vegetation

A uniquely high level of anoxia-tolerance has been observed in Spitsbergen populations of some common high arctic vascular plant species (Crawford *et al.*, 1994a). In this study, portable anaerobe jars were taken to Spitsbergen and intact plants, complete with root systems, were placed on moist filter papers in the jars and kept in the dark at ambient air temperatures for 7 d. The plants were then observed for a further 7 d on return to air for signs of post-anoxic injury. The most surprising aspect of anoxia-tolerance in these species is their ability to maintain turgid, green leaves throughout the period of anoxia and into the post-anoxic recovery phase (Plates 3, 4). Prolonged anoxia-tolerance has never been reported previously for green leaves, which normally lose turgor and wither rapidly when deprived of oxygen. Tests on more

southern populations from Norway, Iceland, and Scotland of species found to be tolerant of anoxia in Spitsbergen (e.g. *Saxifraga oppositifolia*) failed to detect an equivalent ability to survive oxygen deprivation. Figure 5 compares the anoxia-tolerance of grass seedlings raised in the laboratory from two northern species with different geographical origins. Both species show considerable tolerance of anoxia, but the population from the north coast of Alaska (*Deschampsia beringensis*) is considerably more tolerant than the population of *Festuca*
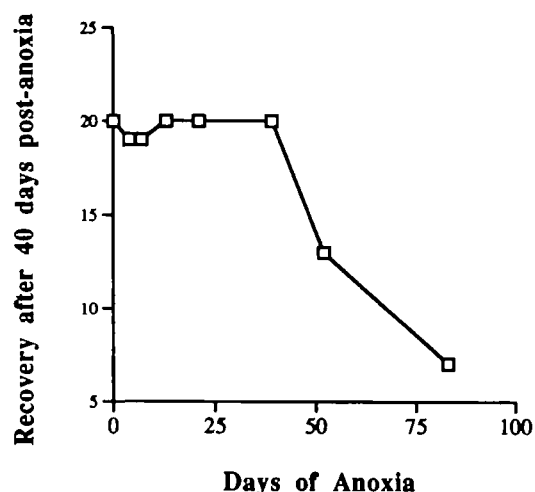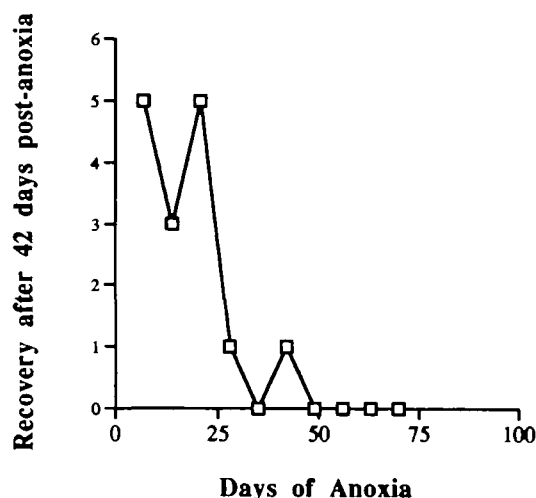
(a) *Deschampsia beringensis*

(b) *Festuca vivipara*

Fig. 5. Comparison of length of viability under anoxia of grass seedlings of (a) an arctic population of *Deschampsia beringensis* Hult. from Alaska and (b) a sub-arctic population of *Festuca vivipara* L. from Iceland. The seedlings were grown in a cold room at 10 °C using seed for *D. beringensis* and clonal pseudo-viviparous plantlets for *F. vivipara*. The y axis records the number of seedlings surviving after allowing a month of post-anoxic recovery following the dark anaerobic incubation at 5 °C.

Flooding Stress

Level 1

| Deprivation of oxygen to plant organs | Production in soil of reduced toxic ions | Changes in plant hormone distribution | Facilitation of microbial pathogens |

Level 2

| Anoxic injury | Post-anoxic injury e.g. oxidative damage to membranes etc | Post-flooding developmental injury e.g. wind throw of trees due to root die-back |

Level 3

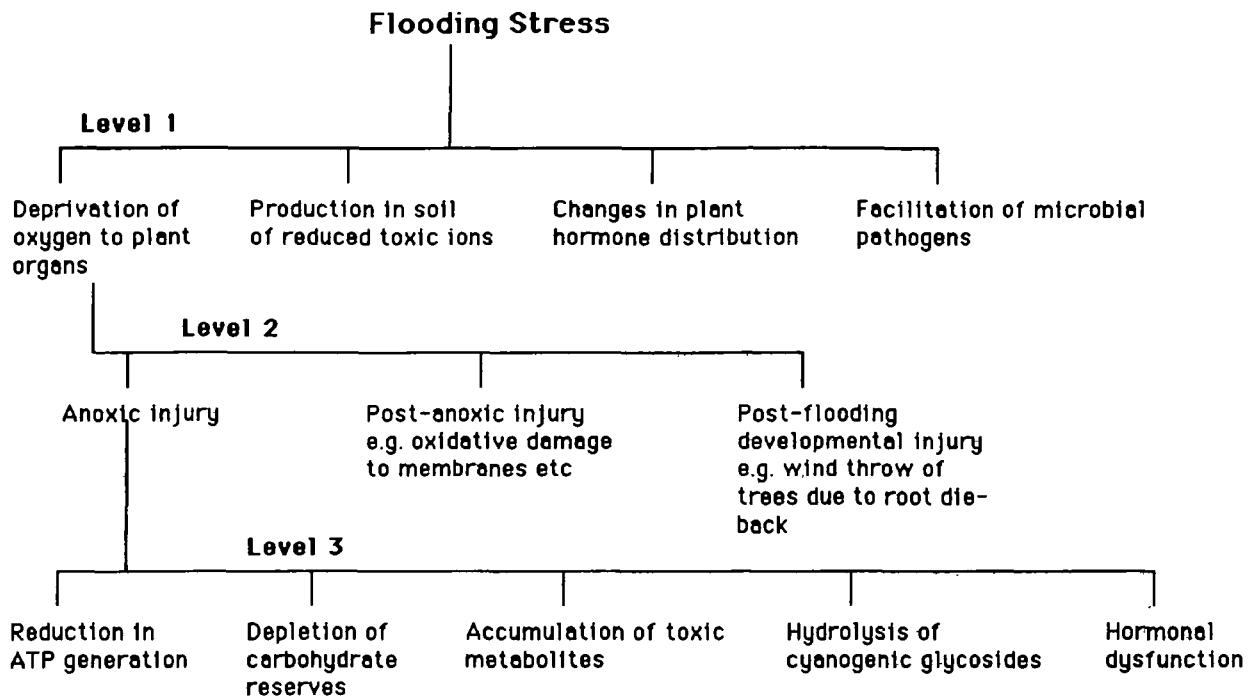| Reduction in ATP generation | Depletion of carbohydrate reserves | Accumulation of toxic metabolites | Hydrolysis of cyanogenic glycosides | Hormonal dysfunction |

Fig. 6. An attempt at segregating the diversity of effects brought about by flooding on higher plants. Level 1 lists four ways in which flooding stress can impinge on plant growth and survival. In level 2 just one of these possible impacts is subdivided, namely deprivation of oxygen into three possible components. In level 3 just one of the level 2 impacts is further subdivided into six possible consequences of anoxic stress.

vivipara from Iceland. This distinctive feature of high arctic populations as compared to more southern populations suggests a different evolutionary history for arctic populations as compared with those from lower latitudes. The greatest frequency of anoxia-tolerant species in Spitsbergen was observed in coastal populations in areas that were prone to winter ice-encasement. Late rains falling on the frozen ground in November in this region freeze immediately and encase the vegetation in a thick layer of ice that usually does not melt until the following summer (June). Thus, with the complete ice-encasement of the tundra not only do the grazing animals starve, the vegetation is completely deprived of any access to oxygen.

Synthesis and conclusions

This review has attempted to illustrate that tolerance of oxygen deprivation is a multi-faceted phenomenon which can respond in a number of ways depending on the nature and duration of the low oxygen stress. Frequently, a reductionist approach to understanding stress in plants seeks for common properties that are shared by a group of plants able to tolerate what appear to be similar adverse conditions. These few divergent flood-tolerance case-histories demonstrate that, under a seemingly simple stress such as oxygen deprivation through flooding, there are many modifying factors which reflect the biodiversity of wetland vegetation (Fig. 6). The distinction between anoxic and post-anoxic injury has important implications

for understanding the effects of flooding on both natural vegetation and crop species. Similarly, the interaction between sulphide and anoxia is fundamental to an understanding of the factors which give rise to reed die-back. In many ways the environment is being slowly and insidiously altered by changes in land use, pollution and climate and, therefore, the nature of flooding and its impact on both wild and cultivated plants can be expected to change. If biodiversity is to be maintained and crop production sustained in areas prone to flooding, there is clearly more that needs to be understood about the divergent consequences of oxygen deprivation on plant survival.

References

Albrecht G, Wiedenroth E-M. 1994. Protection against activated oxygen following re-aeration of hypoxically pretreated wheat roots. The response of the glutathione system. *Journal of Experimental Botany* 45, 449–55.

Alscher RG. 1989. Biosynthesis and antioxidant function of gluathione in plants. *Physiologia Plantarum* 77, 457–64.

Armstrong J, Armstrong G, Beckett PM. 1992. *Phragmites australis:* venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. *The New Phytologist* 120, 197–207.

Armstrong W, Braendle R, Jackson MB. 1994. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica* 43, 307–58.

Barclay AM, Crawford RMM. 1982. Plant growth and survival

under strict anaerobiosis. *Journal of Experimental Botany* 33, 541-9.

Barclay AM, Crawford RMM. 1983. The effect of anaerobiosis on carbohydrate levels in storage tissues of wetland plants. *Annals of Botany* 51, 255-9.

Bergman HF. 1953. Disorders of cranberries. *Plant diseases*. US Department of Agriculture Year Book 789-96.

Bradley PM, Morris JT. 1990. Influence of oxygen and sulphide concentration on nitrogen uptake kinetics in *Spartina alterniflora*. *Ecology* 7, 282-7.

Braendle R. 1990. Flooding resistance of rhizomatous amphibious plants. In: Jackson MB, Davies DD, Lambers H, eds. *Plant life under oxygen deprivation*. The Hague: Academic Publishing, 35-46.

Braendle R, Crawford RMM. 1987. Rhizome anoxia-tolerance and habitat specialization in wetland plants. In: Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*. Oxford: Blackwell Scientific Publications, 397-410.

Brix H. 1989. Gas exchange through dead culms of reed, *Phragmites australis* (Cav.) Trin. ex Steudel. *Aquatic Botany* 35, 99-109.

Bucher M, Braendle R, Kuhlemeier C. 1995. Glycolytic gene expression in amphibious *Acorus calamus* L. under natural conditions. *Plant and Soil* (in press).

Bucher M, Kuhlemeier C. 1993. Long-term anoxia-tolerance. Multi-level regulation of gene expression in the amhibious plants *Acorus calamus* L. *Plant Physiology* 103, 441-8.

Crawford RMM. 1967. Alcohol dehydrogenase activity in relation to flooding in roots. *Journal of Experimental Botany* 18, 454-64.

Crawford RMM. 1989. *Studies in plant survival*. Oxford: Blackwell Scientific Publications.

Crawford RMM. 1992. Oxygen availability as an ecological limit to plant distribution. *Advances in Ecological Research* 23, 93-185.

Crawford RMM, Chapman HM, Hodge H. 1994a. Anoxia-tolerance in high arctic vegetation. *Arctic and Alpine Research* 26, 308-12.

Crawford RMM, Walton JC, Wollenweber-Ratzer B. 1994b. Similarities between post-ischaemic injury to animal tissues and post-anoxic injury in plants. *Proceedings of the Royal Society of Edinburgh* 102B, 325-32.

Crawford RMM, Wollenweber-Ratzer B. 1992. Influence of L-ascorbic acid on post-anoxic growth and survival of chickpea seedlings (*Cicer arietinum* L.). *Journal of Experimental Botany* 43, 703-8.

Eck P. 1990. *The American cranberry*. New Brunswick: Rutgers University Press.

Elstner EF, Osswald W. 1994. Mechanisms of oxygen activation during plant stress. In: Crawford RMM, Hendry GAF, Goodman BA, ed. *Proceedings of the Royal Society of Einburgh*. Edinburgh: The Royal Society of Edinburgh, 131-54.

Fox GG, McCallan NR, Ratcliffe RG. 1995. Manipulating cytoplasmic pH under anoxia: a critical test of the role of pH in the switch from aerobic to anaerobic metabolism. *Planta* 195, 324-30.

Foyer CH, Halliwell B. 1976. The presence of glutathione and glutatione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133, 21-5.

Fürtig K, Ruegsegger A, Brunold C, Braendle R. 1996. Sulphide utilization and injuries in hypoxic roots and rhizomes of reed plants (*Phragmites australis*). *Folia Geobotanica & Phytotaxonomica* (in press).

Grosse W, Büchel HH, Tiebel H. 1991. Pressurized ventilation in wetland plants. *Aquatic Botany* 39, 89-98.

Gudleifsson BE. 1994. Metabolite accumulation during ice encasement of timothy grass (*Phleum pratense* L.). *Proceedings of the Royal Society of Edinburgh* 102B, 373-80.

Gudleifsson BE, Larsen A. 1993. Metabolic and cellular impact of ice encasement on herbage plants. In: Li PH, Christersson L, eds. *Advances in plant cold hardiness*. Boca Raton, Florida: CRC Press, 229-49.

Haldemann C, Braendle R. 1986. Jahrzeitliche Unterschiede im Reservstoffgehalt und von Gärungsprozessen in Rhizomen von Sumpf- und Röhrichtpflanzen aus dem Freiland. *Flora* 178, 307-13.

Haldemann C, Braendle R. 1988. Amino acid composition in rhizomes of wetland species in their natural habitat and under anoxia. *Flora* 180, 407-11.

Hendry GAF, Brocklebank KJ. 1985. Iron-induced oxygen radical metabolism in waterlogged plants. *The New Phytologist* 101, 199-206.

Henzi T, Braendle R. 1993. Long-term survival of rhizomatous species under oxygen deprivation. In: Jackson MB, Black, CR, eds. *Interacting stresses on plants in a changing climate*, NATO ASI Series, Vol. 16. Berlin: Springer-Verlag, 305-14.

Houghton JT, Jenkins GJ, Ephraums JJ (eds). 1990. *Climatic change—the IPCC scientific assessment*. Cambridge University Press.

Huck MG. 1970. Variation in taproot elongation as influenced by composition of soil air. *Agronomy Journal* 62, 815-18.

Jackson MB, Davies DD, Lambers H (eds). 1990. *Plant life under oxygen deprivation*. The Hague, The Netherlands: SPB Academic Publishing.

Jackson MB, Hermann B, Goodenough A. 1982. An examination of the importance of ethanol in causing injury to flooded plants. *Plant, Cell and Environment* 5, 163-72.

Koch M, Mendelssohn IA, McKee KL. 1990. Mechanism for the hydrogen sulphide-induced growth limitation in wetland macrophytes. *Limnology and Oceanography* 35, 399-408.

Koch MS, Mendelssohn IA. 1989. Sulphide as a soil phytotoxin: differential responses in two marsh species. *Journal of Ecology* 77, 565-78.

Koncalova H. 1990. Anatomical adapations to waterlogging in roots of wetland graminoids: limitations and drawbacks. *Aquatic Botany* 38, 127-34.

Kozlowski TT (ed). 1984. *Flooding and plant growth*. New York: Academic Press.

Laan P, Clement JMAM, Blom CWPM. 1991. Growth and development of *Rumex* roots as affected by hypoxic and anoxic conditions. *Plant and Soil* 145, 145-51.

Larson RA. 1988. The antioxidants of higher plants. *Phytochemistry* 27, 969-78.

Lemke-Keyes CA, Sachs MM. 1989. Anaerobic tolerant null: a mutant that allows Adh1 nulls to survive anaerobic treatment. *Journal of Heredity* 80, 316-19.

Monk LS, Fagerstedt KV, Crawford RMM. 1987. Superoxide dismutase as an anaerobic polypeptide. A key factor in recovery from oxygen deprivation in *Iris pseudacorus*. *Plant Physiology* 85, 1016-20.

Ostendorp W. 1987a. Die Auswirkung von Mahd und Brand auf die Ufer-Schilfbestände des Bodensee-Untersees. *Natur und Landschaft* 62, 99-102.

Ostendorp W. 1987b. Damage by episodic flooding to *Phragmites* reeds in a prealpine lake: a proposal of a model. *Oecologia* 86, 119-24.

Ostendorp W. 1993. Schilf als Lebensraum. Beihefte zu den Veröffentlichungen für Natur- und Landschaftspflege in Baden-Würtemberg 68, 173-280.

Pearson J, Havill DC. 1988. The effect of hypoxia and sulphide on culture-grown wetland and non-wetland plants. II.

Metabolic and physiological changes. *Journal of Experimental Botany* **39**, 431–9.

Perata P, Alpi A. 1993. Plant responses to anaerobiosis. *Plant Science* **93**, 1–17.

Perata P, Geshi N, Yamaguchi J, Akazawa T. 1993. Effect of anoxia on the induction of α-amylase in cereal seeds. *Planta* **191**, 402–8.

Perata P, Pozueta-Romero J, Azakawa T, Yamaguchi J. 1992. Effect of anoxia on starch breakdown in rice and wheat seeds. *Planta* **188**, 611–18.

Pezeshki SR, Pan SZ, DeLaune RD, Patrick Jr WH. 1988. Sulphide-induced toxicity: inhibition of carbon assimilation in *Spartina alterniflora*. *Photosynthetica* **22**, 437–42.

Pfister-Sieber M, Braendle R. 1994. Aspects of plant behaviour under anoxia and post-anoxia. *Proceedings of the Royal Society of Edinburgh* **102B**, 313–24.

Powell AA, Matthews S. 1978. The damaging effect of water on dry pea embryos during imbibition. *Journal of Experimental Botany* **29**, 1215–29.

Pretorius JC, Small JGC. 1991. The effect of soaking injury in bean seeds on protein synthesis in embryonic axes. *Seed Science Research* **1**, 195–7.

Roberts JKM, Andrade FH, Anderson IC. 1985a. Further evidence that cytoplasmic acidosis is a determinant of flooding intolerance in plants. *Plant Physiology* **77**, 492–4.

Roberts JKM, Callis J, Jardetzky O, Walbot V, Freeling M. 1984. Cytoplasmic acidosis as a determinant of flooding intolerance in plants. *Proceedings of the National Academy of Science, USA* **81**, 6029–33.

Roberts JKM, Lane AN, Clark RA, Nieman RH. 1985b. Relationships between the rate of synthesis of ATP and the concentrations of reactants and products of ATP hydrolysis in maize root tips, determined by ^{31}P nuclear magnetic resonance. *Archives of Biochemistry and Biophysics* **240**, 712–22.

Saglio PH, Drew MC, Pradet A. 1988. Metabolic acclimation to anoxia by low (2–4 kPa) partial pressure oxygen pretreatment in root tips of *Zea mays*. *Plant Physiology* **86**, 61–6.

Sieber M, Braendle R. 1991. Energy metabolism in rhizomes of *Acorus calamus* (L.) and in tubers of *Solanum tuberosum* (L.) with regard to their anoxia-tolerance. *Botanica Acta* **104**, 279–82.

Studer C, Braendle R. 1987. Ethanol, acetaldehyde, ethylene release and ACC concentration of rhizomes from marsh plants under normoxia, hypoxia and anoxia. In: Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*, Special publication 5, British Ecological Society. Oxford: Blackwell Scientific Publications, 293–301.

Studer C, Braendle R. 1988. Postanoxische Effekte von Äthanol in Rhizomen von *Glyceria maxima* (Hartm.) Holmberg, *Iris germanica* (Cav.) Trin. *Botanica Helvetica* **98**, 111–21.

Studer-Ehrensberger K, Studer C, Crawford RMM. 1993. Flood-induced change in a dune slack observed over 24 years. *Functional Ecology* **7**, 156–68.

Ushimaru T, Shibasaka M, Tsuji H. 1992. Development of O_2^- detoxification system during adaptation to air of submerged rice seedlings. *Plant and Cell Physiology* **33**, 1065–71.

Van Toai TT, Bolles CS. 1991. Post-anoxic injury in soybean (*Glycine max*) seedlings. *Plant Physiology* **97**, 588–92.

Weber M, Braendle R. 1994. Dynamics of nitrogen-rich compunds in roots, rhizomes and leaves of the sweet flag (*Acorus calamus*). *Flora* **189**, 63–8.

Weber M, Braendle R. 1996. Some aspects of the extreme anoxia-tolerance of the sweet flag (*Acorus calamus*) L. *Folia Geobotanica and Phytotaxonomica* (in press).

Wollenweber-Ratzer B, Crawford RMM. 1994. Enzymatic defence against post-anoxic injury in higher plants. *Proceedings of the Royal Society of Edinburgh* **102B**, 381–90.